RESEARCH ARTICLE

Potentiality of Novel Chitosan-O-Arginine as Insecticidal and Growth Inhibitory Compound against Diamondback Moth, *Plutella xylostella* L. on Cauliflower

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ABSTRACT

Chitosan (Cs), a natural biodegradable polysaccharide polymer, prepared from sea waste of crustacean origin, has the potential for plant protection application. In the present study, a novel chitosan derivative, called Chitosan-O-Arginine, synthesized from crude chitosan, was evaluated for its chronic toxicity against second instar larvae of *Plutella xylostella* by leaf dip method. The results revealed the toxic potential of Chitosan-O-Arginine, which could inflict cent per cent mortality after four days of continuous feeding on the 1000 ppm treated leaf, and the larva could accomplish 0.12 mg weight (99.66% reduction) on the day before death. The next lower concentration (700 ppm) resulted in the maximum larval weight of 0.45 mg (93.71% reduction) after nine days of feeding and resulted in cent per cent mortality after the tenth day. While the untreated larva attained a maximum weight of 5.54 mg. All the larvae were pupated in the next lower concentration (500 ppm) and recorded minimum pupal (1.00 mg; 80% reduction) and adult (0.73 mg; 77.45% reduction) weight, whereas the untreated pupa and adult weighed the maximum of 5.00 mg and 3.20 mg. Considering the developmental period, there was no significant difference in the larval duration and adult life span. While there was an extension of the pupal period by one day at 500 ppm compared to untreated larva. The larval, pupal, and adult malformations were noticed in 300 and 100 ppm concentrations. These findings suggest that chitosan was potent in imposing chronic toxicity and growth inhibitory effect on *P. xylostella* larvae, and hence it can be recommended as an eco-friendly component in the Integrated Pest Management module.

Keywords: *Plutella xylostella; Chitosan-O-Arginine; Chronic toxicity; Growth inhibition; Malformations*

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae) is a widespread pest of cultivated and wild Brassicaceae *viz*., cabbage, cauliflower, broccoli, Brussels sprouts, radish and field crops such as turnip, mustard, and rape. *P. xylostella* can cause a high yield loss of 91.2% (Elizeu *et al.*, 2020). Using insecticides at higher rates cause many detrimental effects such as residue, resurgence, resistance and environmental hazards. All field populations exhibited high levels of resistance against chlorantraniliprole (36.67 to 124.72 fold), flubendiamide (12.66 to 93.63 fold), fipronil (31.99 to 156.24 fold), and cypermethrin

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(26.97 to174.84 fold) (Tamilselvan *et al*., 2021). This has necessitated the identification and use of alternative eco-friendly insecticides to manage diamondback moth. Recently, chitosan is gaining momentum in plant protection; hence, this research has a major focus.

The marine waste of chitinous solid available in India ranges from 60,000 to 80,000 tons per annum. The composition of chitin varied depending on the aquatic animal, it was reported that dry prawn waste, dried squilla, dry wastes of shrimp and crab processing wastes contain 23, 15, 14-27 and 13-15 per cent chitin, respectively (Madhavan, 1978; Ibrahim *et al*., 1991; Subasinghe, 1995). Ibrahim *et al*. (1991) reported that the seafood processing industry is using shrimp as a significant raw material (45%), which contain exoskeleton to the extent of 30-40% by weight and is discarded as waste. If they are not disposed of properly, they may cause pollution and pose a significant threat to the environment. These wastes are converted into valid and economically worthy materials, *viz*., chitin and chitosan. This conversion helps improve the economic potential of this waste into a by-product during food processing. It also helps to minimize the potential threat as a source of environmental pollution. The use of this innovative renewable waste material into economically appreciable and sustainable materials facilitates the conversion of waste to wealth, which would open up an untapped market.

Chitosan is one of the most abundant natural amino polysaccharides extracted from the exoskeleton of crustaceans and insects, from fungal cell walls, *etc*. (Katiyar *et al*., 2014). The term chitosan is used when the polymers become soluble in a dilute acid solution. The solubility is also controlled by the distribution of the acetyl groups remaining along the chain (Matteus, 1997). Chitosan may serve as an excellent alternative to pest control because of its insecticidal and antimicrobial properties (Rabea *et al*., 2003 and Badawy *et al*., 2005). The chitosan exhibited the highest insecticidal effect against lepidopteran and homopteran insect pests. In addition, it displayed higher increased insecticidal activity against *P. xylostella* than on *Helicoverpa armigera* and *Spodoptera exigua*. It was reported to be effective against sucking insect pests like aphids *Hyalopterus prun* (Zhang *et al*., 2003). Sayed *et al.* (2014) reported that chitosan was an active insecticide against fourth instar larvae of *Spodoptera littoralis* with mortality of 92.19 per cent at 5000 ppm concentration. The insect mortality can be achieved relatively at its low dosage levels, and they are non-toxic to vertebrates and humans. Chitosan treatments are effective against herbivorous insect pests, but it is being used successfully as an ingredient in the artificial diet fed to carnivorous insects being reared for the biological control of chitinous pests (Tan *et al*., 2010). With this background, the present study focused on synthesizing novel chitosan derivative called Chitosan-O-arginine, characterized and evaluated its toxicity and antifeedant potential against diamond back moth, *P. xylostella*.

MATERIALS AND METHODS

The synthesis and evaluation were conducted in the Natural Pesticide Laboratory, Department of Agricultural Entomology, Agriculture College and Research Institute, Tamil Nadu Agricultural University, Madurai, during 2020 -2021.

Synthesis of Chitosan-O-arginine (CS-O-Arg)

The chitosan-O-arginine (CS-O-Arg) was synthesized by adopting the method suggested by Hefni *et al*. (2020). Initially, the crude chitosan flakes were suspended in 100 mL of distilled water. Later, the acid derivative L - arginine was added to the chitosan solution (at the ratio 1: 2). The catalyst, sulfuric acid (H_2SO_4) was added to the chitosan arginine solution at ambient temperature. L - arginine was sparingly soluble in H_2SO_4 solution, while adding 5 mL HCl increased the solubility of L - arginine. The mixture was stirred at 80 °C using a condenser to prevent vaporization. The chitosan-O-arginine derivatives were precipitated by adjusting the pH to neutral. Then the precipitate was filtered and washed with acetone, and it was designated as CS-O-Arg.

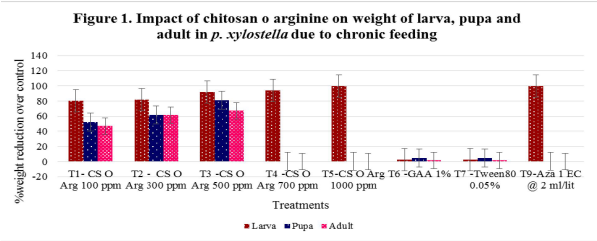
Soxhlet Purification with Soxhlet Apparatus

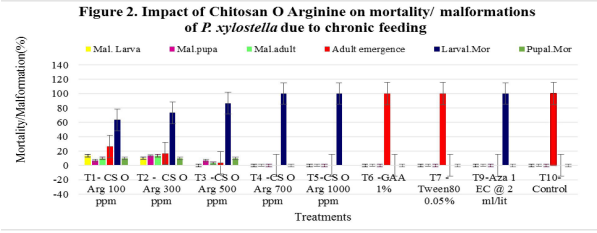
The precipitate of Chitosan-O arginine derivatives was extracted using acetone in the Soxhlet apparatus. Later the residue was oven-dried, sieved, and stored in the refrigerator until further use for bioassay.

Mass culturing of *P. xylostella*

Diamondback moth *P. xylostella* larvae were collected from infested cauliflower at the vegetable market in Madurai, Tamil Nadu. Newly hatched caterpillars were confined in 8 L plastic containers,

covered with cotton (gada) cloth on the top. Inside the container, fresh and clean cauliflower leaves with stalks wrapped in moist cotton served as a feeding substrate and were replaced every day until caterpillars reached the pupal stage (Ruiz *et al*., 2021). Pupae were kept in Petri dishes, inside an insect rearing cage (1.5x1.5x1.5 ft), and after emergence, adults were fed with 10 per cent honey solution soaked in cotton. Cauliflower leaves were offered as an oviposition substrate, and eggs were removed once every two days and placed in plastic pots until the caterpillars hatched.





Effect of Chitosan-O-Arginine on *P. xylostella*

The effect of Chitosan-O-Arginine on the growth and development of *P. xylostella* larvae was estimated by chronic exposure. In the case of the chronic feeding test, the larvae were fed with treated food from second to final instar. The Chitosan-O-Arginine was dissolved in the solvent, 1% glacial acetic acid and mixed with surfactant, Tween 80 0.05% and prepared at different concentrations *viz*., 100 ppm, 300 ppm, 500 ppm, 700 ppm and 1000 ppm. Tween 80 0.05%, 1% glacial acetic acid were kept as negative checks, Azadirachtin 1% EC @ 2 mL/L was used as treated check, in comparison with untreated check. The evaluation was done by leaf bits dip bioassay. The leaf bits (4x4 mm) were prepared from young cauliflower leaves leaving the hard veins and used for the bioassay. Each treatment was replicated thrice and for each replication, 10 larvae were released. Larval weight was recorded daily. Pupal and adult weights were also recorded. The per cent reduction in weight of larva, pupal and adult over control was estimated. If there were any malformations or mortalities during any life stages in different treatments, that were also recorded. Larval, pupal period, adult life span and number of adults emerged were recorded. Per cent adult emergence was estimated (Tian *et al*., 2020).

Data Analysis

All the experiments were conducted under Completely Randomized Block Design (CRBD). Data were statistically analyzed using SPSS for Windows (version 22) (IBM Corp. Released 2013) software to carry out ANOVA. Grouping of data was done using Tukey's HSD (Honestly Significant Difference) test (Tukey, 1977).

RESULT AND DISCUSSION

Synthesis of Chitosan-O-Arginine

The Chitosan-O-Arginine prepared from the raw material, called crude chitosan, to which the L - arginine was added, which increased their mass by three times and the recovery yield was 70.96%. The raw chitosan was in the form of flakes, and it was transformed into Chitosan-O-Arginine form after acylation process. Earlier, the crude chitosan did not possess aqueous solubility, but the synthesis of Chitosan-O-Arginine helped in enhancing their solubility in 1% aqueous glacial acetic acid. In addition, the water binding capacity and Degree of Deacetylation (%) were also improved in Chitosan-O-Arginine compared to crude chitosan (Selvarani *et al*., 2021). As the crude chitosan is insoluble in water, which is a major hurdle in its applications, it was transformed into chitosan-O- Arginine by acylation process with L - arginine, in the presence of sulphuric acid as catalyst and hydrochloric acid as solubility agent for arginine, to increase the water solubility and biological activity.

Chronic feeding effect of Chitosan-O-Arginine on growth and development of *P. xylostella*

Tests focused on chronic toxicity, determined that Chitosan-O-Arginine was significantly more efficient compared to control. The inhibition effect of the Chitosan-O-Arginine on *P.xylostella* larval growth is shown in Table 1. Larval growth was inhibited at all the concentrations of Chitosan-O-Arginine. Nevertheless, important differences were noticed. When comparing the weight of larva on the third day after treatment (D7), it was minimum in 1000 ppm (0.12 mg) and azadirachtin 1 % EC @ 2 mL/L (0.04 mg) and both were statistically on par. It was followed by Chitosan-O-Arginine 700 ppm (0.34 mg), while, untreated check had 1.19 mg and maximum

larval weight attained in these, chitosan-O-Arginine 300 ppm treatments were 0.92 mg and 5.40 mg per larva, respectively.

As shown in Table 2, Chitosan-O-Arginine significantly affected larval, pupal and adult weight, and they were very minimum in Chitosan-O-Arginine 500 ppm treatment. The larval weight gain was very low in 1000 and 700 ppm Chitosan-O-Arginine (0.01 and 0.34 mg, respectively) compared to control larval weight gain (5.43 mg). Correspondingly, the pupal and adult weight was also reduced to 500 ppm (1.00 mg and 0.73 mg, respectively), while the untreated check recorded the maximum pupal and adult weight (5.00 mg and 3.20 mg, respectively). This finding disclosed that the per cent reduction in larval, pupal, and adult weight in 500 ppm Chitosan-O-Arginine treatment over untreated check was 91.71%, 80.01% and 77.45%, respectively (Figure 2). The pupal period of *P. xylostella* was prolonged by one day, when fed with Chitosan-O-Arginine 500 ppm treated leaves (6 days) compared to untreated check (5 days) (Table 2). In case of pupal period and adult life span, no significant difference was found among the treatments.

From the chronic feeding study, it was found that 1000 ppm, 700 ppm of Chitosan-O-Arginine and azadirachtin 1 % EC caused 100 per cent larval mortality on fourth day after treatment (Figure 1). While 500 ppm Chitosan-O-Arginine caused larval mortality of 86.66 per cent and 300 ppm Chitosan-O-Arginine caused malformations of larva, pupa and adult to an extent of 10.00, 13.33 and 3.33 per cent, respectively (Figure 1). At the same time, there was no mortality of larva, pupa and adult in untreated check. These results confirmed that Chitosan-O-Arginine at 1000 and 700 ppm were effective in causing mortality and inhibiting the growth of *P. xylostella.*

The efficacy of the chitosan-O- Arginine in terms of chronic toxicity on *P. xylostella* was clearly established and 1000 ppm of Chitosan-O-Arginine caused cent per cent larval mortality on the fourth day after treatment. *P. xylostella* larval growth inhibition by Chitosan-O- Arginine was also demonstrated in this study.

Table 1. Impact of Chitosan- O- Arginine (CS-O- Arg) on larval weight of *P. xylostella* due to chronic feeding

Treatment	Mean fresh weight of the larvae (mg)#							
	D4	D5	D6	D7	D8	D9	D10	D11
T1 – CS-O-Arg 100 ppm	0.11±0.01 (0.33)^{a}	0.19±0.02 (0.44)^{c}	0.28±0.01 (0.53)^{d}	0.54±0.02 (0.74)^{d}	0.68±0.02 (0.83)^{c}	0.87±0.01 (0.9)^{d}	0.92±0.02 (0.96)^{c}	1.18±0.03 (1.09)^{b}
T2 – CS-O-Arg 300 ppm	0.10±0.01 (0.31)^{a}	0.18±0.01 (0.42)^{bc}	0.28±0.01 (0.53)^{d}	0.44±0.02 (0.66)^{c}	0.66±0.05 (0.81)^{c}	0.73±0.03 (0.9)^{c}	0.84±0.03 (0.91)^{c}	1.09±0.10 (1.04)^{b}
T3 – CS-O-Arg 500 ppm	0.11±0.01 (0.33)^{a}	0.16±0.01 (0.41)^{bc}	0.21±0.01 (0.45)^{c}	0.44±0.01 (0.66)^{c}	0.55±0.03 (0.74)^{b}	0.57±0.02 (0.8)^{b}	0.41±0.02 (0.64)^{b}	0.71±0.03 (0.84)^{a}
T4 – CS-O-Arg 700 ppm	0.11±0.01 (0.33)^{a}	0.15±0.01 (0.39)^{b}	0.16±0.01 (0.40)^{b}	0.34±0.02 (0.58)^{b}	0.44±0.03 (0.66)^{a}	0.45±0.01 (0.26)^{a}	0.16±0.01 (0.40)^{a}	Dead
T5 – CS-O-Arg 1000 ppm	0.10±0.01 (0.32)^{a}	0.08±0.01 (0.28)^{a}	0.06±0.01 (0.24)^{a}	0.12±0.01 (0.35)^{a}	Dead	Dead	Dead	Dead
T6 – Acetic acid 1%	0.11±0.01 (0.32)^{a}	0.35±0.01 (0.59)^{d}	0.56±0.01 (0.75)^{e}	1.20±0.01 (1.10)^{e}	2.03±0.01 (1.43)^{d}	4.15±0.07 (2.04)^{e}	5.36±0.2 (2.31)^{d}	5.31±0.30 (2.30)^{c}
T7 – Tween 80 0.05%	0.11±0.01 (0.32)^{a}	0.35±0.01 (0.59)^{d}	0.56±0.01 (0.75)^{e}	1.20±0.06 (1.90)^{e}	2.09±0.05 (1.44)^{d}	4.16±0.09 (2.04)^{e}	5.40±0.05 (2.32)^{d}	3.51±3.04 (1.87)^{c}
T8 – Azadirachtin 1% EC @ 2 mL/L	0.10±0.01 (0.32)^{a}	0.06±0.01 (0.24)^{a}	0.11±0.01 (0.33)^{a}	0.04±0.04 (0.20)^{a}	Dead	Dead	Dead	Dead
T9 – Untreated check	0.10±0.01 (0.32)^{a}	0.35±0.03 (0.59)^{d}	0.57±0.01 (0.75)^{e}	1.19±0.02 (1.09)^{e}	2.10±0.01 (1.45)^{d}	4.22±0.05 (2.07)^{e}	5.54±0.08 (2.35)^{d}	5.13±0.23 (2.27)^{c}
S.Ed	0.005 ^{NS}	0.012*	0.007*	0.022*	0.021*	0.036*	0.093*	0.83*
F value	0.819	160.18	1095.85	131.30	3106.67	7875.88	1339.80	13.06
P value	0.596	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Mean values of three replications are represented as mean ± standard deviation; Figures in the parentheses are square root transformed values (√x+0.5); the mean followed by the same letter are not significantly different from each other by Tukey's test ($p \leq 0.05$); S.Ed: Standard Error of the difference; NS- Non significant ; *- Significant.

Table 2. Impact of Chitosan-O-Arginine on development of *P. xylostella* due to chronic feeding on treated leaf

Treatments	Average larval period (days)$^{-1}$	Average pupal period (days)$^{-1}$	Average adult longevity(days)$^{-1}$
T_1 – CS-O-Arg 100 ppm	11.00±0.00 (3.31)^{a}	5.00±0.00 (2.23)^{bc}	15.33±0.57 (3.91)^{a}
T_2 – CS-O-Arg 300 ppm	11.00±0.00 (3.31)^{a}	5.33±0.57 (2.30)^{b}	15.00±0.00 (3.87)^{a}
T_3 – CS-O-Arg 500 ppm	10.66±0.57 (3.26)^{a}	6.00±0.00 (2.44)^{a}	15.33±0.57 (3.91)^{a}
T_6 – Acetic acid 1%	10.66±0.57 (3.26)^{a}	5.00±0.00 (2.23)^{c}	15.33±0.57 (3.91)^{a}
T_7 – Tween80 0.05%	10.33±0.57 (3.21)^{a}	5.00±0.00 (2.23)^{c}	15.00±0.00 (3.87)^{a}
T_9 – Untreated check	11.00±0.00 (3.31)^{a}	5.00±0.00 (2.23)^{c}	15.00±0.00 (3.87)^{a}
S.Ed	0.36 ^{NS}	0.25**	0.36 ^{NS}

#Mean values of three replications are represented as mean ± standard deviation; Figures in the parentheses are square root transformed values (√x+0.5); the mean followed by the same letter are not significantly different from each other by Tukey' s test ($p \leq 0.05$); S.Ed: Standard Errors of the difference; NS – non significant; **- Highly significant

Earlier, Zhang *et al.* (2003) reported that commercial oligo-chitosan at 3 g per lit (3000 ppm) was an active insecticide against *P. xylostella* with 72% mortality after 72 hours of treatment might be the formation of a film on the surface of insects. Thus, the film could block air for respiration insects, and ultimately, the insect died. Similar findings have been reported for 5000 ppm of commercial chitosan, which caused 92% oral mortality of *S. littoralis* after 72 hours of treatment (Sayed *et al*., 2014). In another study, chitosan derivative (N-(2-chloro-6-fluorobenzyl) was found effective against S. littoralis with an LC_{50} of 0.32 g kg^{-1} diet and 100% mortality at ≥0.625 g kg^{-1} (Badawy *et al*., 2012).

In addition, higher toxicity of chitosan-metal complexes was observed on *S. litura* with (97.3%) different molecular weights. The insecticidal activity of chitosan complexes against another insect *Aphis nerii* was 84.4% mortality after 48 h (Rabea *et al.,* 2005). Typically, from the first day of feeding on the treated leaf, chitosan-O-Arginine inhibited the larval growth in time dependent manner in this study. When topically applied on the larval body, commercial chitosan (Sigma) scored 52.51% of *S. litura* larval mortality with 3.0 g/ L (3000 ppm) at seven days after treatment, which might be a cause for higher action of chitosan as well as for the highest larval mortality and growth inhibition of larvae (Uddin *et al.*, 2021). An earlier study of N-alkyl chitosan (NAC) derivatives against *S. litura* reported that, it strongly inhibited the larval weight gain. The insect growth was significantly decreased and the normal ecdysis process was affected. They exhibited symptoms like inhibition of feeding, weight gain, and the larvae were very small compared with the controls (Rabea *et al*., 2006). Moorthy *et al.* (2021) reported that seven per cent of colloidal chitosan caused 85.38 per cent antifeedant effect against first instar larva of *Spodoptera frugiperda* and from the first day of feeding on the treated leaf, this colloidal chitosan inhibited the larval growth in a time-dependent manner.

Conclusion

Chitosan-O-Arginine was toxic against *P. xylostella* after continuous feeding on the treated leaves and inhibited the growth and development of *P. xylostella*, which resulted in weight reduction in larva, pupa and adult and also prolonged the larval developmental period at higher concentration. Hence, it demonstrated that Chitosan-O-Arginine has the insecticidal and growth inhibitory potential on diamond back moth, *P. xylostella*. It needs to be further validated through field experiments.

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Ethics Statement

Specific permits were not required for the above studies because no human or animal subjects were involved in this research.

Originality and plagiarism

The authors declare that the work carried out in this research paper is the original work carried out and has not been published earlier or sent for publication to other research journals.

Consent for publication

All the authors agreed to publish the content.

Competing interests

The authors declare that they have no competing interests.

Data Availability

All the data of this manuscript are included in the manuscript. No separate external data source is required.

Author's contribution

MS, MM, KS, SV and SH performed the idea of this article. SS wrote the manuscript. MS participated in writing the manuscript and statistical analysis. MS and MM contributed the material and helped in the maintenance of *P. xylostella*, while all authors equally contributed in conducting bioassay experiments. The authors read and approved the final manuscript.

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